MASS. EA1.2: EN89 Executive Office of Environmental Affairs

MASSACHUSETTS DEPARTMENT OF NATURAL RESOURCES

ENVIRONMENTAL IMPACT STATEMENT

DRAFT

Suppression of Gypsy Moths in an Area Wide Application of Encapsulated Sex Attractant

April 23, 1973

Government Documents Collection
AUG 1973
University of Massachusetts

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Statement:

1. Description - The gypsy moth, a serious pest of trees in the northeastern United States, continues to threaten the Nation's hardwood forests. Gypsy moth caterpillars strip the leaves from approximately 400,000 acres of timber a year, (from about 1,900,000 and 1,260,000 acres in 1971 and 1972 respectively). This damage coupled with necessary control measures is very costly. A native of Europe, the gypsy mtoh, was accidentally introduced into America in 1869, when imported specimens escaped from the laboratory of a Medford, Massachusetts naturalist. Without natural enemies to contain it, the moth spread rapidly and now is threatening to spread to states throughout most of the Eastern seaboard. It is a serious pest in the State of Massachusetts.

The gypsy moth caterpillars feed on the leaves of forest, shade, and fruit trees as well as on many ornamentals. A single defoliation by caterpillars can kill white pine, spruce, and hemlock. Two defoliations can kill hardwoods. In heavily infested areas, caterpillars can quickly strip the leaves from whole forests. This damage in turn, increases fire and erosion hazards, affects the flow of streams, reduces land and recreational values, and destroys wildlife habitats.

Crawling caterpillars are a nuisance in residential and recreational areas. Since 1906 the States and the U.S. Department of Agriculture have been working together to control and prevent the spread of the gypsy moth. This pest is currently being controlled by costly and time-consuming applications of insecticides over large areas of forests in the infested states.

Publication No.(6814-50-6-73-CR)

With the recent development of a sex pheromone, disparlure, an alternative method for control appears promising. This chemical (cis - 7,8 - epoxy - 2 - mehtyloctadecane) will be used to divert or confuse males in their search to find females for mating and propagation (confusion method).

A search was made for an area in Massachusetts suitable for a test application of the pheromone. Major consideration was given to the finding of an area of at least 25 square miles with a gypsy moth population of from 0-10 egg masses per acre.

The treatment area is located in the north-central part of the State and includes parts of the towns of Erving, Northfield and Warwick. This is a heavily wooded area sparsely populated by human beings. The southern border of the project is just north of Route#2. It is bounded on the east by Wendall Road, on the west by Route #63 and the north by White Road in Warwick and the Stratton Mountain Road in Northfield.

The gypsy moth has been established in this area for approximately 50 years. Records indicate a somewhat regular rise and fall in populations. The last major outbreak in this area, and part of an outbreak extending from the western edge of Worcester County to the Berkshires, was in 1962 and 1963. In 1963 the Department of Natural Resources aerially sprayed 110,000 acres with DDT in the Connecticut Valley Region, including the 25 square mile block in qestion. From that period up to the present, gypsy moth populations in the test block have been insignificant.

The proposed testing of an aerial application of disparlure in July of 1973 is the culmination of a series of smaller tests conducted over the past two years. The Entomology Research Division of the Agricultural Research Service, U.S.D.A feels that this test will finally prove or disprove the theory that a gypsy moth population can be depressed following an application of the synthetic sex attractant.

Use of the confusion mehtod would involve a single application by aircraft of two to four grams of the sex attractant per acre. The sex attractant will be formulated in microcapsules for slow sustained release of the lure. A sticker will be added to fasten the microcapsules to foliage thereby securing vertical stratification of the lure in the forest canopy; also less lure will reach the forest floor where debris and other matter may inhibit the release of the sex attractant into the atmosphere. The formulation of the sex attractant will also contain a thickener to keep particles in suspension during spraying.

Disparlure appears to be innocuous based on the results of (1) acute and oral toxicity tests on albino rats (LD_{50}greater than 34,600 mg/kg); (page 2, enclosure #1) (2) acute dermal toxicity tests (LD_{50}greater than 2,025 mg/kg; (page 2, eclosure #1), (3) eye irritation tests; (page 2, enclosure #1), (4) primary skin irritation tests; (page 2, enclosure #1), (5) inhalation tests; (page 2, enclosure #2).

2. Environmental impact - If the results of this test indicate that the gypsy moth populations have been reduced significantly, birds feeding on these insects may be deprived some food. However, it is not expected that this deprivation will have an adverse effect on bird populations since there is no known species of birds that depends on gypsy moths as its sole food source. Since the moth flight usually occurs only for about two weeks, normal dependence of birds on the moths for food is short-lived. Furthermore, use of the pheromone will be limited to low population infestations, and should not affect the total food reservoir of birds appreciably. The natural enemies of the gypsy moth, including parasites and predators, may by reduced because of lack of suitable host material. However, it is not anticipated that this reduction in the numbers of beneficial natural control agents will adversely effect the environment since the primary insect predators and parasites have only recently been

introduced into the United States.

The sprays of encapsulated disparlure also represent an environmental contaminate; however, so little is used that it is extremely difficult to find any of the capsules after they are dispensed. (Probably no more than 2 g of capsular material/acre will be used). Accordingly, no noticeable effects on the environment, including those on plants, fish, wildlife, are anticipated. No change in land use, water, or public services in treated areas is expected. Economic impacts on employment, unemployment, and other economic factors would be nil. The resort areas are presently suffering from the moth's depredations. Eventually the moth could invade commercial forests and the economic impact resulting from insufficient timber could be substantial.

3. Favorable enviromental effects - If the gypsy moth is controlled, it would prevent damage to the trees in the test area. This would prevent the destruction of many hardwood trees, white pine, spruce, and hemlock. These trees in turn, provide a favorable habitat for birds and other wildlife. Thereby, control of the gypsy moth would prevent the destruction of the habitats of these important species. If the destruction of the trees is prevented, then we have prevented the erosion of soil and subsequent silting of streams, thus avoiding possible harm to fish and other aquatic wildlife. Preventing the destruction of these forest lands increases their recreational values and timber resource potential. What is perhaps more important is that the spread of the moth will be halted, and areas outside the infested one will remain free of the moth until we can develop a suitable means to control or eliminate the insect.

4. Adverse environmental effects which cannot be avoided -- There will be some contamination of the environment due to exhaust fumes from vehicles and aircraft used in the area to conduct the study. However, the emission of these air pollutants from the operation of the vehicles in these areas would be of short duration and would appear to have only a minimal effect on the ecosystem.

There will be a slight loss of food to birds when gypsy moths are reduced in number, but it is believed that the percentage of total volume in the diet of birds of gypsy moths is quite small.

Temporary dispersion of wildlife may be expected because of the noise created by the vehicles and aircraft. However, it is anticipated that the wildlife will return to their normal habitats when work cree leave the area.

5. Alternatives -.

A. Do nothing: The present damage incurred every year from gypsy moth caterpillars defoliating trees would continue, and the moth will continue to spread. Trees adversely affected from the defoliations may die and subsequently wildlife habitats would be reduced. Eventually, commercial forests would be hit with periodic severe infestations. We would not gain any knowledge concerning the probability of using alternative methods to control the gypsy moth. Any delay in stopping the spread of the moth will result in additional areas becoming infested and the task of preventing the spread of the moth will become much more costly and difficult.

B. Use of insecticides: This would be a continuation of our present methods to control the gypsy moth in forested areas, which would result in a greater environmental impact, on a continuing basis, than if we could develop alternative methods of control.

C. Use of natural enemies and disease: Natural enemies and disease help reduce populations of gypsy moths, but their action is not predictable or controllable at this time, and these agents have not prevented destruction of large areas of forests. The cost of propagating natural enemies and diseases of the gypsy moth for release and control, even if successful, would far exceed the cost of the proposed method. Biological control has great appeal, but much more research must be done before it can be considered a realistic alternative. Finally, biological control, if successful, will minimize damage in the infested

areas, but it will not prevent the spread of the gypsy moth throughout its potential range, which includes forests in the western U.S.

6. Relationship between local short-term uses of man's environment and the maintenance and enhancement of long-term productivity - If this project is successful, it can be broadened to include the entire gypsy moth infested area. Areas not infested will not be subject to the moth's depredations. If insecticide applications can be curtailed, the economic benefits could be substantial. In additions, the overall condition of the environment will be upgraded rather than degraded.

7. Irreversible and irretrievable commitment of resources - None.

dustrial BIO-TEST Laboratories, Inc.

REPORT TO

UNITED STATES DEPARTMENT OF AGRICULTURE

ACUTE TOXICITY STUDIES WITH DISPARLURE

P.O. NO. RB-19685-ARS-72

OCTOBER 20, 1972

IBT NO. A1958

I. Introduction

A sample identified as Disparlure (cis-7,8-epoxy-2-methyloctadecane) was received from the United States Department of Agriculture. The following studies* were conducted:

Acute Oral Toxicity Study - Albino Rats

Acute Dermal Toxicity Study - Albino Rabbits

Eye Irritation Test - Albino Rabbits

Primary Skin Irritation Test - Albino Rabbits

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* Federal Insecticide, Fungicide and Rodenticide Act

Industrial **BIO-TEST** *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

#1

October 20, 1972

Dr. Morton Beroza, Leader
Organic Chemical Synthesis
Laboratory, Agricultural
Environmental Quality Institute
United States Department of Agriculture
Beltsville, Maryland 20705

Dear Dr. Beroza:

Re: IBT No. A1958 - Acute Toxicity Studies with
Disparlure - Purchase Order No. RB-19685-ARS-72

We are submitting herewith our laboratory report dated October 20, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

cc: Dr. P. H. Schwartz

Publication No. (6815-50-28-73-CR)

II. Summary

The results of the acute toxicity studies with Disparlure (cis-7, 8-epoxy-2-methyloctadecane) are summarized below with respect to F.I.F.R.A. classification.

Test	Classification
Acute Oral Toxicity Study - Albino Rats	Slightly Toxic ($LD_{50} > 34,600$ mg/kg)
Acute Dermal Toxicity Study - Albino Rabbits	Slightly Toxic ($LD_{50} > 2,025$ mg/kg)
Eye Irritation Test - Albino Rabbits	Not an Irritant (12.0/110.0)
Primary Skin Irritation Test - Albino Rabbits	Not an Irritant (2.8/8.0)

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by:

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III. Investigational Procedures

A. Acute Oral Toxicity Study - Albino Rats

Young albino rats of the Sprague-Dawley strain* were used as test animals. All animals were kept under observation for five days prior to experimental use, during which period they were checked for general physical health and suitability as test animals. The animals were housed in stock cages and were permitted a standard laboratory diet plus water *ad libitum*, except during the 16-hour period immediately prior to oral intubation when food was withheld.

Initial screening was conducted in order to determine the general level of toxicity of the test material. Selected groups of four albino rats each (two males and two females) were administered the test material at several dose levels. All doses were administered directly into the stomachs of the rats using a hypodermic syringe equipped with a ball-tipped intubating needle.

After oral administration of the test material, the rats were housed individually in suspended, wire-mesh cages and observed for the following 14 days. Initial and final body weights and reactions were recorded. A necropsy was conducted on all animals sacrificed at the end of the 14-day observation period.

* ARS/Sprague-Dawley, Madison, Wisconsin.

At the end of the observation period, the acute oral median lethal dose (LD_{50}) of the test material was determined. The test material was then assigned a classification in accordance with the Federal Insecticide, Fungicide and Rodenticide Act. The classification system is:

Acute Oral LD_{50} (Range of Values)	Classification
$\leq$ 50 mg/kg	Highly Toxic
> 50 mg/kg but $\leq$ 500 mg/kg	Toxic
> 500 mg/kg	Slightly Toxic

B. Acute Dermal Toxicity Study - Albino Rabbits

Young adult albino rabbits of the New Zealand strain were used as test animals. All rabbits had been maintained under observation in the laboratory for at least seven days prior to testing. During the pre-test period, the animals were examined with respect to their general health and suitability as test animals. The rabbits were housed individually in suspended, wire-bottomed cages and maintained on a standard laboratory ration. Food and water were offered ad libitum.

Twenty-four hours prior to the dermal applications, the backs of the rabbits were shaved free of hair with electric clippers. The shaved area on each animal constituted about 30 percent of the total body surface area. The animals were then returned to their cages to await testing on the following day. The 24-hour waiting period allowed recovery of the stratum corneum from the disturbance which accompanied the close-clipping procedure and permitted healing of any microscopic abrasions possibly produced during the process.

The test material was applied at a dose level of 2,025 mg/kg. Four rabbits (two males and two females) were tested. The test site was covered by wrapping the trunk of the animal with impervious plastic sheeting which was securely taped in place. This plastic wrap insured close contact of the epidermis and test material. To prevent oral ingestion of the test material, each animal was fitted with a lightweight, flexible plastic collar which was worn throughout the observation period.

The test material remained in contact with the skin for 24 hours. At the end of this period, the plastic sheeting and all residual test material were removed. The test sites were examined for local skin reactions and the animals were returned to their cages. Observations for mortality, local skin reactions, and behavioral abnormalities were continued for a total of 14 days following the skin applications. Initial and final body weights were recorded. A necropsy was conducted on all animals sacrificed at the end of the observation period.

After the completion of the study, the test material was classified in accordance with the Federal Insecticide, Fungicide and Rodenticide Act. The classification system is:

Acute Dermal LD_{50} (Range of Values)	Classification
$\leq$ 200 mg/kg	Highly Toxic
> 200 $\leq$ 2,000 mg/kg	Toxic
> 2,000 mg/kg	Slightly Toxic

C. Eye Irritation Test - Albino Rabbits

Young albino rabbits of the New Zealand strain were used to evaluate the eye irritating properties of the test material.

Exactly 0.1 ml of the undiluted sample was instilled into the conjunctival sac of the right eye of each rabbit according to the treatment procedure presented in Table I. The left eye of each animal served as a scoring control. The maximum possible score at any one examination and scoring period is 110 points which indicates maximal irritation and damage to all three ocular tissues. Zero score indicates no irritation. The Draize scoring system is presented in Table II.

After the completion of the test, the scores were analyzed and the test material classified as a Corrosive, Irritant, or Not an Irritant. The criteria used for assignment of the descriptive rating were the frequency, the extent, and the persistence of irritation or damage which occurred to the three ocular tissues. The classification system is presented in Table III.

TABLE I

Eye Irritation Test - Albino Rabbits

Treatment Procedure

Test Material	Number of Animals Evaluated	Form Administered	Quantity of Test Material Administered	Contact Period (seconds)	Volume of Wash (tap water)	Scoring Intervals
Disparlure	6	Undiluted	0.1 ml	Unlimited	None	One, 24, 48, and 72 hours, and 7 days

TABLE II

Eye Irritation Test - Albino Rabbits

Scale of Weighted Scores for
Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Grading
Cornea	Opacity (A)	
	Opacity - Degree of density (area which is dense is taken for reading).	
	Scattered or diffuse area, details of iris clearly visible.	1
	Easily discernible translucent areas, details of iris slightly obscured.	2
	Opalescent areas, no details of iris visible, size of pupil barely discernible	3
	Opaque, iris invisible	4
	Area of Cornea Involved (B)	
	One quarter (or less) but not zero	1
	Greater than one-quarter but less than one-half.	2
	Greater than one-half but less than three-quarters.	3
	Greater than three-quarters, up to whole area.	4
	Score equals A x B x 5 Total maximum = 80	
Iris	Values (A)	
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive).	1
	No reaction to light, hemorrhage, gross destruction (any or all of these).	2
	Score equals A x 5 Total maximum = 10	

TABLE II continued

Eye Irritation Test - Albino Rabbits

Scale of Weighted Scores for Grading the Severity of Ocular Lesions

Ocular Tissues	Description	Grading
Conjunctiva	Redness (A)	
	Redness (refers to palpebral conjunctiva only).	
	Vessels definitely injected above normal.	1
	More diffuse, deeper crimson red, individual vessels not easily discernible.	2
	Diffuse beefy red.	3
	Chemosis (B)	
	Any swelling above normal (includes nictitating membrane).	1
	Obvious swelling with partial eversion of the lids.	2
	Swelling with lids about half-closed.	3
	Swelling with lids about half-closed to completely closed.	4
	Discharge (C)	
	Any amount different from normal (does not include small amount observed in inner canthus of normal animals).	1
	Discharge with moistening of the lids and hairs just adjacent to the lids.	2
	Discharge with moistening of the lids and hairs and considerable area around eye.	3
	Score (A + B + C) x 2 Total maximum = 20	

Note: The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctiva.

TABLE III

Eye Irritation Test - Albino Rabbits

Classification System

Descriptive Rating	Criteria
Corrosive	The structure of the tissue at the site of contact is destroyed or changed irreversibly in 24 hours or less.
Irritant	Four or more animals in a test group of six animals exhibit a positive reaction. An animal shall be considered as exhibiting a positive reaction if the test substance produces at 24, 48, or 72 hours any of the following reactions: I. Cornea A. Ulceration other than a fine stippling B. Opacity with a density of grade 2 II. Iris with a grade 2 reaction (2 x 5 = 10) III. Conjunctiva with redness (grade 2) or swelling (grade 2)
Not an Irritant	No irritation or slight irritation (grade 1 reaction)

Note: A Draize score of 2 or more for any of the parameters and tissues under consideration is equivalent to a positive reaction as defined in the Federal Insecticide, Fungicide and Rodenticide Act.

D. Primary Skin Irritation Test - Albino Rabbits

Young albino rabbits of the New Zealand strain were used in the evaluation of the primary skin irritating properties of the test material. The test procedure was modeled after that of Draize et al.*

Prior to the application of the test material, the hair was clipped from the back and flanks of each rabbit. Two test sites located lateral to the midline of the back approximately ten centimeters apart were selected. One of the two sites was abraded by making four epidermal incisions, two perpendicular to the other two, while the other test site remained intact.

Exactly 0.5 ml of undiluted test material (0.5 g of test material moistened with a minimum amount of water in the case of solids) was applied to each of the test sites on each rabbit. The test sites were immediately occluded with two-inch square gauze patches. The patches were placed directly over the test sites and secured with masking tape. The trunk of each animal was then wrapped with impervious plastic sheeting. The wrap held the patches in position and retarded evaporation of the test material during the 24-hour exposure period.

At the end of 24 hours, the plastic wrappings and patches were removed. The intact and abraded test sites were examined and scored separately for erythema and edema on a graded scale of 0 to 4. After 72 hours, the sites were reexamined and rescored. The scoring criteria for erythema and edema are presented in Table IV.

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* Draize, John H., Woodard, Geoffrey, and Calvery, Herbert O., "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," J. Pharm. & Exp. Ther.

TABLE IV

Primary Skin Irritation Test - Albino Rabbits

Scoring Criteria for Skin Reactions

Reaction	Description	Score
Erythema	Barely perceptible (Edges of area not defined)	1
	Pale red in color and area definable	2
	Definite red in color and area well defined	3
	Beet or crimson red in color	4
Edema	Barely perceptible (Edges of area not defined)	1
	Area definable but not raised more than 1 mm	2
	Area well defined and raised approximately 1 mm	3
	Area raised more than 1 mm	4
Injury in Depth	Escharosis, Necrosis	8
	Maximum Primary Irritation Score =	8

In evaluating the average irritation present, the mean scores for erythema and edema of the intact test sites after 24 and 72 hours were added. Similarly, the mean scores for erythema and edema of the abraded test sites after 24 and 72 hours were added. These two values were totaled and divided by four to obtain the mean primary irritation score. The test material was then classified as a Corrosive, Irritant or Not an Irritant. The classification system is presented in Table V.

TABLE V

Primary Skin Irritation Test - Albino Rabbits

Classification System

Descriptive Rating	Criteria
Corrosive	The intact skin of the albino rabbit is destroyed or changed irreversibly in 24 hours or less.
Irritant	Primary irritation score is equal to or greater than 5.
Not an Irritant	No irritation or primary irritation score is less than 5.

IV. Results

A. Acute Oral Toxicity Study - Albino Rats

1. Mortality and Body Weights

Individual mortality and body weight data are presented in Table VI.

TABLE VI

TEST MATERIAL: Disparlure

Acute Oral Toxicity Study - Albino Rats

Mortality and Body Weight Data

Dose* (mg/kg)	Animal Number and Sex	Individual Body Weights (grams) Test Day Number: 0	14	Number Dead / Number Tested	Percent Dead
10,250	1-M	224	333	0/4	0
	2-M	228	365		
	3-F	206	237		
	4-F	200	232		
15,380	5-M	230	312	0/4	0
	6-M	230	340		
	7-F	173	220		
	8-F	169	211		
23,070	9-M	223	346	0/4	0
	10-M	229	358		
	11-F	225	268		
	12-F	201	226		
34,600	13-M	237	360	0/4	0
	14-M	237	354		
	15-F	222	253		
	16-F	195	237		

Acute Oral $LD_{50} > 34,600$ mg/kg

* The test material was administered undiluted.

2. Reactions

The untoward reactions exhibited by the rats following oral administration of Disparlure are presented in Table VII.

Necropsy revealed no gross pathologic alterations among any of the animals.

TABLE VII

TEST MATERIAL: Disparlure

Acute Oral Toxicity Study - Albino Rats

Summary of Reactions

Dose (mg/kg)	Reaction	Time of Onset Following Dose Administration	Duration of Reaction	Time of Death Following Dose Administration
10,250	Hypoactivity	2 hours	3 days	-
	Ruffed fur	2 hours	3 days	
	Diuresis	6-22 hours	2 days	
15,380	Hypoactivity	2 hours	3 days	-
	Ruffed fur	2 hours	3 days	
	Diuresis	6-22 hours	2 days	
23,070	Hypoactivity	2 hours	3 days	-
	Ruffed fur	2 hours	3 days	
	Diuresis	6-22 hours	2 days	
34,600	Hypoactivity	2 hours	3 days	-
	Ruffed fur	2 hours	3 days	
	Diuresis	6-22 hours	2 days	

B. Acute Dermal Toxicity Study - Albino Rabbits

1. Mortality and Body Weights

Individual mortality and body weight data are presented in Table VIII.

TABLE VIII

TEST MATERIAL: Disparlure

Acute Dermal Toxicity Study - Albino Rabbits

Mortality and Body Weight Data

Dose* (mg/kg)	Animal Number and Sex	Individual Body Weights (kg) Test Day Number:		Number Dead / Number Tested	Percent Dead
		0	14		
2,025	1-M	2.66	2.48	0/4	0
	2-M	2.64	2.45		
	3-F	2.40	2.30		
	4-F	2.46	2.10		

Acute Dermal $LD_{50} > 2,025$ mg/kg

* The test material was applied undiluted.

2. Reactions

No untoward behavioral reactions were noted among any of the animals dermally exposed to Disparlure.

Local skin reactions were characterized at the end of the 24-hour contact period by red, well-defined erythema and severe edema (area raised more than 1 mm). Escharosis, desquamation, hemorrhaging, and fissures were noted after 7 days. Desquamation, fissures, and pustules were observed after 14 days.

Necropsy of all rabbits sacrificed at the end of the 14-day observation period did not reveal any gross pathologic alterations other than the dermal alterations as previously described.

C. Eye Irritation Test - Albino Rabbits

The results of the eye irritation test are presented in Table IX.

TABLE IX

TEST MATERIAL: Disparlure

Eye Irritation Test - Albino Rabbits

Results

Tissue	Rabbit Number	1 Hour	24 Hours	48 Hours	72 Hours	7 Days
Cornea (D-A)	1	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	4 (2-0-0)	0	0	0
Total		12	4	0	0	0
Cornea (D-A)	2	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	0	0	0	0
Total		12	0	0	0	0
Cornea (D-A)	3	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	2 (1-0-0)	0	0	0
Total		12	2	0	0	0
Cornea (D-A)	4	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	4 (2-0-0)	0	0	0
Total		12	4	0	0	0

TABLE IX continued

TEST MATERIAL: Disparlure

Eye Irritation Test - Albino Rabbits

Results

Tissue	Rabbit Number	1 Hour	24 Hours	48 Hours	72 Hours	7 Days
Cornea (D-A)	5	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	0	0	0	0
Total		12	0	0	0	0
Cornea (D-A)	6	0	0	0	0	0
Iris		0	0	0	0	0
Conjunctiva (R-S-D)		12 (2-1-3)	0	0	0	0
Total		12	0	0	0	0
Averages						
Cornea		0.0	0.0	0.0	0.0	0.0
Iris		0.0	0.0	0.0	0.0	0.0
Conjunctiva		12.0	1.7	0.0	0.0	0.0
Total		12.0	1.7	0.0	0.0	0.0

Cornea:
D = Density
A = Area
Corneal Score = D x A x 5
Maximum Score = 80

Iris:
Iris Score = Value x 5
Maximum Score = 10

Conjunctiva:
R = Redness
S = Swelling
D = Discharge
Conjunctival Score = (R+S+D) x 2
Maximum Score = 20

D. Primary Skin Irritation Test - Albino Rabbits

The results of the primary skin irritation test are presented in Table X.

TABLE X

TEST MATERIAL: Disparlure

Primary Skin Irritation Test - Albino Rabbits

Results

Animal Number	Irritation Scores for Abraded Skin Sites At: 24 Hours Er.	Ed.	72 Hours Er.	Ed.	Irritation Scores for Intact Skin Sites At: 24 Hours Er.	Ed.	72 Hours Er.	Ed.
1	2	1	2	1*	2	1	2	1*
2	2	1	2	0	2	1	2	0
3	2	0	2	0*	2	0	2	0*
4	2	1	2	1*	2	1	2	1*
5	2	1	2	1*	2	1	2	1*
6	2	1	2	1*	2	1	2	1*
Mean	2.0	0.8	2.0	0.7	2.0	0.8	2.0	0.7
Subtotal		5.5				5.5		

Primary Irritation Score = 2.8

Key:

Er. = Erythema
Ed. = Edema

* Desquamation

#2

Industrial BIO-TEST *Laboratories, Inc.*
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

October 30, 1972

Dr. Morton Beroza, Leader
Organic Chemical Synthesis
Laboratory, Agricultural
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United States Department of Agriculture
Beltsville, Maryland 20705

Dear Dr. Beroza:

Re: IBT No. A1958 - Acute Aerosol Inhalation Toxicity
Study with Disparlure in Albino Rats
Purchase Order No. RB-19685-ARS-72

We are submitting herewith our laboratory report dated October 30, 1972, prepared in connection with the above study.

Very truly yours,

J. C. Calandra
President

JCC/kjl

cc: Dr. P. H. Schwartz

Publication No. (6816-50-9-73-CR)

REPORT TO

UNITED STATES DEPARTMENT OF AGRICULTURE

ACUTE AEROSOL INHALATION TOXICITY STUDY WITH DISPARLURE IN ALBINO RATS

P.O. NO. RB-19685-ARS-72

OCTOBER 30, 1972

IBT NO. A1958

I. Introduction

A sample identified as Disparlure was received from the United States Department of Agriculture for the purpose of conducting an acute aerosol inhalation toxicity study using albino rats as experimental animals.

II. Summary and Results

Ten albino rats were exposed to an aerosol of Disparlure for a one-hour period in a 70-liter inhalation chamber. The average nominal aerosol concentration was calculated to be 5.0 mg/L air. This was the maximum respirable aerosol concentration attainable with the experimental equipment employed. After exposure all the animals were observed for 14 days.

No deaths were noted during the one-hour exposure period or 14-day observation period which followed. Disparlure is classified as non-toxic.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Report prepared by: James R. Grapenthien
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III. Procedure

The procedure followed was in accordance with the evaluation of inhalation toxicity specified in the Federal Regulations*, Section 191.1 (f) (2).

A group of ten albino rats was exposed for one hour to an aerosol of the test material. The test was conducted in a Plexiglas inhalation chamber having a volume of 70 liters. The chamber was designed so that the animals could be introduced to the test atmosphere after the maximum aerosol concentration was established. Each animal was caged separately during exposure to minimize filtration of inspired air by animal fur. Following exposure, the animals were observed for a period of 14 days.

An aerosol of the undiluted test material was generated with a pneumatic nebulizer capable of producing liquid particles in a size range of one to ten microns. The average nominal aerosol concentration, calculated by dividing the nebulizer weight loss by the total volume of air used during the test, was 5.0 mg/L air at 29.92 inches Hg and 25°C. This was the maximum respirable aerosol concentration attainable with the experimental equipment employed.

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* Code of the Federal Regulations, Part 191, Chapter I, Title 21, reprinted from Federal Register of August 12, 1961; 26 F.R. 7333.

Additional Informati
herein

1. (Chemistry of the pesticides, properties, etc.)

cis-7,8-Epoxy-2-methyloctadecane is a sex attractant emitted by the non-flying female gypsy moth to lure the male to it for mating. The synthetic compound is called disparlure.

Physical properties: colorless liquid; $n_D^{28} = 1.4435 \pm 0.0010$; solubility, 0.15 ppm in water at 25°C, soluble in organic solvents; volatility at 25°C, slightly less than that of methyl palmitate; infrared spectrum attached.

The material used is at least 96% pure, as determined by gas chromatography (retention time 4.2 min. on a 12-ft-long, 1/4-inch o.d. column containing 5% Carbowax on 60/80 mesh Gas Chrom P; carrier gas, helium at 60 ml./min.) It contains no more than 15% of the trans isomer, as determined by GC on a Carbowax 20M support coated open tubular column.

Biological properties and field performance: Reprints are enclosed giving data on biological properties and field trials.

Method of synthesis: Disparlure is prepared by epoxidation of (Z)-2-methyl-7-octadecene with m-perchlorobenzoic acid or peracetic acid. The olefin precursor can be synthesized by a Wittig reaction or via the acetylene. Synthesis of disparlure is described on p. 663 of the attached article by Bierl, Beroza, and Collier (J. Econ. Entomol. 65, 659-665 (1972)). Directions for an alternative synthesis of the olefin precursor and for epoxidation with peracetic acid are also attached.

2. (Chemistry of the formulation, properties of each ingredient)

Disparlure, as a 1 to 3 % solution in xylene in gelatine-base or nylon-base microcapsules, is sprayed as an aqueous slurry. The aqueous phase of the formulation contains a thickener to keep the capsules in suspension during spraying and a sticker to hold the capsules on foliage.

Ingredients: Capsule slurry--Disparlure

Xylene, a solvent, has been exempted from tolerance requirements when used as a solvent for pesticide formulations used on growing crops (Pesticide Chemical News, Jan. 17, 1973)

Microcapsule wall - gelatine, which may be coated with a paint, or nylon-base plastic

Thickener--Hydroxyethyl cellulose (Soilserve Minidrift, Union Carbide), Federally exempt (under 180.001) from

residue tolerance requirements. See attached pamplet on Cellosize.

Potassium hydroxide, an alkali to bring about hydration of the hydroxyethyl cellulose for thickening action.

Sticker--Ucar Latex 680. (Union Carbide). A styrene-acrylic copolymer, total solids content 46%, with water as carrier. Stabliized by soaps (prime stabliizer, ammonium oleate).

Residual monomer concentration, less than 0.4%.

Average particle size, 0.2 µ. Density 1.02 g/cc.

pH 9. Viscosity, 50 centipoise. Surface tension

after dilution to 30% solids, 36 dynes/cm.

4. (Quantity to be shipped, name and address of manufacturer(s), and shipper.) About 9000 kg of formulation (for treatment of ~~40~~ 25 sq. miles). National Cash Register Co., 1000 Cox Plaza, 3131 South Dixie Drive, Dayton, Ohio 45439 is manufacturer of microcapsules. USDA will formulate the microcapsules with sticker and thickener just prior to use.

5. (Description of proposed use program)

Disparlure is to be used as a "confusant" to control the gypsy moth, Porthetria dispar (L.). By permeating the air with disparlure, male moths are prevented from locating females for mating in areas of light infestation (up to 2000 insects/sq. mile). (In our most recent tests, the "confusant" was effective for about 6 weeks.) The major test will be conducted in a forest area totalling ~~40~~ 25 square miles or less.

Disparlure in a slow-release formulation (microcapsules) will be applied by aircraft at a rate of 2 g/acre. The formulation will include a sticker to fasten the microcapsules to leaves and a thickener to keep particles in suspension during spraying.

The number of male moths captured in the test area and in an untreated control area by traps baited with live sterile female moths will be determined. Females under bark flaps will also be exposed in the treated and untreated areas to determine whether males can find them. At the end of the test, females will be checked to see if they have been mated. Effectiveness of treatment will be judged by the ability of the formulation to prevent males from finding the females in the test area. Egg mass counts before and after the test may also be made.

6. Time of testing will be between May 15 and October 1973.

The major test with disparlure will be conducted during moth flight, which occurs once a year in midsummer and generally lasts 2-3 weeks. The formulation will be applied about a week before the anticipated beginning of moth emergence (about July 15, 1973).

Pre-season and post-season tests of very small magnitude by comparison (less than 1/2 square mile) will also be conducted with experimental formulations.

7. (States in which program will be conducted) Unknown. Sites to be selected.

9. (Method of analysis)

Disparlure is analyzed by gas chromatography using a flame ionization detector. Attached specifications provide analytical method.

8. (Proposed labeling--pesticide name, name and address of manufacturer or registrant,net content, ingredient statement)

Disparlure (cis-7,8-epoxy-2-methyloctadecane)
   Gypsy moth confusant formulation
      USDA-ARS
   Contents: disparlure 5g/kg, inert ingredients 99.5% in an aqueous suspension.

3. (Confidential formula - composition may be varied)

Capsule slurry: 182 kg total weight. Contains: 54.4 kg microcapsules slurried in water

Microcapsules contain solution of 2.2% disparlure in xylene

Total disparlure 1090 g.

Thickener: 78.04 kg of 1% hydroxyethyl cellulose in water to which is added 4.55 kg 1% potassium hydroxide in water

Sticker: 5.35 kg Ucar Latex 680

Mixing: Thickener and sticker to be added just before spraying

Total Weight: 270 kg.

Coverage: The 270 kg is enough for 545 acres. At this rate, the amount applied per acre will be 494 ml formulation containing 100 g microcapsules which contain 2 g disparlure.

Industrial **BIO-TEST** *Laboratories, Inc.*
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1800 EAST PERSHING ROAD
DECATUR, ILLINOIS 62526

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AREA CODE 217
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November 8, 1972

Mr. Morton Beroza, Leader
Organic Chemical Synthesis
Laboratory, Agricultural
Environmental Quality Institute
United States Dept. of Agriculture
Beltsville, Maryland 20705

Dear Mr. Beroza:

Enclosed are the results of four-day static fish toxicity studies for P.O. RB-19685-ARS-72.

Please do not hesitate to call if there are any questions.

Sincerely,

John W. Goode, Ph.D.

John W. Goode, Ph.D.
Manager
Decatur Research Laboratories

JWG/dm
enc. (3)

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REPORT TO
UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
AGRICULTURAL ENVIRONMENTAL QUALITY INSTITUTE
RESULTS OF FOUR-DAY STATIC FISH TOXICITY STUDIES RAINBOW TROUT AND BLUEGILLS

P. O. RB-19685-ARS-72

IBT NO. A-1958

Test Material: Disparlure
Description: Clear, oily liquid
Form Administered: 1.0% and 10.0% (w/v) solution in acetone

Concentration of Test Material (ppm)	Parameter	Rainbow Trout Elapsed Time (hours) 1-6	24	48	72	96	Total	Bluegills Elapsed Time (hours) 1-6	24	48	72	96	Total
100.0	pH	-	-	-	6.6	-	-	-	-	-	-	-	-
	DO	-	-	-	2.3	-	-	-	-	-	-	-	-
	°C	-	-	-	12.6	-	-	-	-	-	-	-	-
	Survivors	10	10	10	8	8	8	10	10	10	10	10	10
10.0	pH	-	-	-	-	-	-	-	-	-	-	-	-
	DO	-	-	-	-	-	-	-	-	-	-	-	-
	°C	-	-	-	-	-	-	-	-	-	-	-	-
	Survivors	10	10	10	10	10	10	10	10	10	10	10	10
1.0	pH	-	-	-	-	-	-	-	-	-	-	-	-
	DO	-	-	-	-	-	-	-	-	-	-	-	-
	°C	-	-	-	-	-	-	-	-	-	-	-	-
	Survivors	10	10	10	10	10	10	10	10	10	10	10	10
0.1	pH	-	-	-	-	-	-	-	-	-	-	-	-
	DO	-	-	-	-	-	-	-	-	-	-	-	-
	°C	-	-	-	-	-	-	-	-	-	-	-	-
	Survivors	10	10	10	10	10	10	10	10	10	10	10	10
Four-Day TL_{50}		> 100.0 ppm						> 100.0 ppm					

Comments:

Prepared by: Gary Rausina
Gary Rausina, B.S.
Group Leader

Approved by: John W Goode
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Manager